

In Situ Monitoring with Tradescantia around Nuclear Power Plants

by Sadao Ichikawa*

Highly sensitive mutational responses of the stamen-hair system of some *Tradescantia* clones heterozygous for flower color (blue/pink, the blue being dominant) to low-level radiation and chemical mutagens, as demonstrated in the last decade, seem to endorse this system to be the most promising biological tester for detecting the genetic effects of mutagens at low levels. Two triploid (thus sterile) clones, KU 7 and KU 9, have been established as those suitable for *in situ* monitoring of environmental mutagens. *In situ* monitoring with such *Tradescantia* clones was first tried in 1974 around a nuclear power plant in Japan, then has been repeated until 1979 around more nuclear plants. About 260,000 to 1,570,000 stamen hairs were observed per year per nuclear plant (about 12-million hairs in total), and the data of pink mutation frequency were analyzed statistically. Significantly increased mutation frequencies were observed and were correlated to the operation periods of the nuclear facilities and to predominant wind direction, but not to other environmental factors. Considering physical monitoring data of radiation dose in the air, internal exposure due to incorporation and concentration of man-made radioactive nuclides seemed to be of a greater importance in increasing mutation incidence.

Introduction

The stamen-hair system of some specific clones of *Tradescantia* has been demonstrated to be an excellent botanical test system to study the genetic effects of physical and chemical mutagens (1-9). Those clones are all heterozygotes for flower and stamen color (blue/pink; the blue color being dominant), and have been established at Brookhaven National Laboratory (clones 02, 2031 and 4430) or at Kyoto University (clones KU 7, KU 9 and KU 20). The characteristics of the *Tradescantia* stamen-hair system, that is, the capability of detecting all pink mutant cells easily without being concealed by other cell (see Fig. 1) as well as the relative ease of handling a great number of samples, have proved to be especially suitable for studying the genetic effects of low-level radiation (7, 8, 10-16) and even the variation of spontaneous mutation frequency (17, 18).

Ichikawa (12, 13) demonstrated that the somatic pink mutation frequency in the stamen hairs kept a linear relationship with chronic gamma-ray and the scattering radiation exposures down to 0.72 R,

after repeating experiments in a gamma field. Sparrow et al. (14) demonstrated further that the somatic pink mutation frequency in *Tradescantia* stamen hairs increased linearly with increasing acute x-ray dose in the extremely small-dose range of 0.25 to 6 rad. According to them, the mutation frequency with 0.43-MeV fast neutrons was linear down to 0.01 rad. Increased mutation frequencies at higher natural background radiation levels (10, 11) and with man-made radioactive nuclides at low levels (15, 16) have been also reported. The dose response curve of the somatic pink mutations has proved to be linear not only in small-dose range (14) but also at low exposure rate (12, 13, 19).

These fundamental data suggest that *Tradescantia* stamen-hair system can be the most promising biological tester for detecting the genetic effects of low-level radiation or radioactivity. The present paper describes the cases in which *in situ* monitoring of radioactive release from nuclear facilities was tried with the stamen-hair system of *Tradescantia*.

Materials and Methods

The materials used were two triploid clones ($2n=18$) of *Tradescantia* heterozygous for flower color, KU 7 and KU 9, both established as those

*Laboratory of Genetics, Department of Regulation Biology, Faculty of Science, Saitama University, Urawa 338, Japan.

suitable for *in situ* monitoring. The KU 7 clone of *T. ohiensis* Raf. (= *T. reflexa* Raf.) has been described to be a tetraploid clone (4, 12, 13, 18) but is reported here as a triploid, since our recent chromosome counts on the stock plants as well as on the materials used in the present study have proved that all of those examined cytologically have 18 chromosomes. The morphological characteristics of this clone are, however, essentially unchanged from those described earlier (4). The KU 9 is a hybrid clone between *T. ohiensis* and *T. paludosa* And. et Woods. (5). Both clones are vigorous in growth and are sterile because of their triploid nature. These characteristics are especially important for the material to be used in outdoor experiments, for obtaining securely a certain number of healthy flowers daily for a long period of time, and for preventing being contaminated by any genetic segregants.

On comparing KU 7 and KU 9 clones, however, the latter which was found later has characteristics feasible for easier observation, namely, a higher contrast between the normal blue and mutant pink colors, shorter hairs (ca. 20 cells per hair) suitable for easier and more accurate scoring, and a greater average number of hairs (ca. 90) per stamen essential for obtaining a larger sample size (5), and a lower spontaneous pink mutation frequency (ca. 3 mutant events per 1000 hairs) (18) desirable for making the background "noise" smaller to secure a more sensitive statistical analysis. Therefore, the KU 7 clone which had been used in earlier years of this study was later substituted by KU 9.

The first long-term scoring of somatic pink mutations in the stamen hairs of KU 7 clone was carried out in July to October of 1974 around the Hamaoka Nuclear Power Plant built at Hamaoka, Shizuoka, before and during its test operation (20). The scoring at this area was continued until 1977, in May to October, every year. Similar long-term scorings were started in 1976 around the Shimane Nuclear Power Plant at Kashima, Shimane, and the Takahama Nuclear Power Plant at Takahama, Fukui. In 1978, the experiments were further expanded to start scorings around the Ohwi Nuclear Power Plant at Ohwi, Fukui, and around the nuclear power complex in Tokai, Ibaraki. Excepting the use of KU 7 at Shimane and Takahama in 1976, KU 9 has been used as the material in these areas (see Table 1).

At any of those areas, the tester plants potted in 24 cm clay pots with soil from the same source were placed at three to nine different points (only one point in case of Ohwi) around the nuclear facility and also at one to three points well apart from the facility. All such points were selected considering

the wind direction, sunlight, drainage, other environmental factors, and the convenience of collecting flowers daily. The plants were watered every day (except rainy days) and were supplied with nutritional solution (Hyponex, 1/1000 solution) once a week.

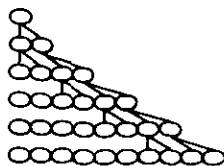
Three to six (mostly four) flowers were collected from each point every early morning and were stored in refrigerator until being observed. For scoring pink mutations, six stamens were taken from each flower, placed in a small amount of liquid paraffin dropped on a slide glass, and were observed under a stereoscope equipped with the Olympus' horseshoe-shaped white-color fluorescent lamp at a magnification of 16 or 20 times. The numbers of stamen hairs and of pink mutant events were recorded for each stamen observed. A pink mutant event is defined as a single pink cell or two or more contiguous pink cells which are considered to have been derived from a single mutation (2) (Fig. 1). In case of finding of two or more entirely pink hairs from a stamen, they were scored as a single mutant event. Also, a row of pink cells in a hair but separated by a single blue cell was regarded as one mutant event, considering the occurrence of a somatic crossing over to be more likely than simultaneous occurrences of two independent mutations. The frequency of pink mutations was expressed as the number of pink mutant events per 1000 hairs. About 260,000 to 1,570,000 stamen hairs were observed per year per nuclear plant, and the total number of stamen hairs observed exceeded 12 million (Table 1).

The above scorings of mutations were carried out by high-school biology teacher (Hamaoka), university students (Shimane), a civil servant (Takahama), a devoted citizen (Ohwi), or a group of high-school teachers and scientists (Tokai), under the guidance of the author. All of those persons involved had been trained in advance for the stamen-hair observation at least for one week.

The data collected were grouped into different points of placing tester plants and into different periods of scoring (mostly every two-week period), and were subjected to a contingency chi-square test and to an analysis of variance. In cases of experiments in which mutation scoring was made by two or more persons, differences between scorers were also examined statistically. Mutation frequency calculated for each point and period was compared with that at control point(s) in the same period and with, if available, the frequency determined at respective point before the operation of the nuclear plant, in order to examine any statistical difference.

Meteorological data employed for the evaluation of mutation data were mostly supplied by local

NORMAL CELL INCREASE



LOSS OF REPRODUCTIVE INTEGRITY



(Often Giant-Cell Formation)

MUTATION IN TERMINAL CELL

Chromosome Type



MUTATION IN SUBTERMINAL CELL

Chromosome Type



EXCEPTIONAL CASES

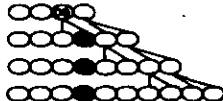


(Division of Interstitial Cell)

Chromatid Type (A)



Chromatid Type (A)



(Terminal Cell Stopped Division)

Chromatid Type (B)



Chromatid Type (B)



(Partial Recovery)

FIGURE 1. Illustrations of the patterns of cell increase and the appearance of mutations and other radiation effects in the stamen hairs of *Tradescantia*.

Table 1. Summary of the *in situ* monitoring experiments with *Tradescantia* around nuclear plants reported in the present paper.

Nuclear plant	Clone	Year	Scoring period	No. of hairs	No. of mutant events	No. of mutant events/1000 hairs
Hamaoka	KU 7	1974	7/7-10/31	637,614	2,778	4.36 ± 0.08
		1975	5/11-10/25	612,487	3,064	5.00 ± 0.09
		1976	5/19-10/31	823,457	3,530	4.29 ± 0.07
		1977	5/31-10/31	675,430	2,685	3.98 ± 0.08
		(Total)		2,748,988	12,057	4.39 ± 0.04
Shimane		1976	6/30-9/29	491,914	1,925	3.91 ± 0.09
Takahama		1976	7/5-10/23	397,101	1,871	4.71 ± 0.11
(Subtotal)				3,638,003	15,853	4.36 ± 0.03
Shimane	KU 9	1977	6/12-10/18	1,102,568	2,613	2.37 ± 0.05
		1978	5/21-9/6	651,481	1,160	1.78 ± 0.05
		1979	6/10-10/15	598,685	1,378	2.30 ± 0.06
		(Total)		2,352,734	5,151	2.19 ± 0.03
Takahama		1977	5/9-10/1	1,318,271	3,989	3.03 ± 0.05
		1978	5/1-10/1	1,569,291	4,001	2.55 ± 0.04
		1979	5/21-9/30	1,203,875	2,513	2.09 ± 0.04
		(Total)		4,091,437	10,503	2.57 ± 0.03
Ohwi		1978	5/1-9/8	623,428	1,751	2.81 ± 0.07
		1979	5/21-9/14	587,772	1,862	3.17 ± 0.07
		(Total)		1,211,200	3,613	2.98 ± 0.05
Tokai		1978	6/12-9/17	261,631	286	1.09 ± 0.06
		1979	5/23-10/20	452,776	828	1.83 ± 0.06
		(Total)		714,407	1,114	1.56 ± 0.05
(Subtotal)				8,369,778	20,381	2.44 ± 0.02
Total				12,007,781	36,234	3.02 ± 0.02

weather stations, but in some cases obtained from local governmental offices in charge of watching nuclear facilities.

Results

Experiments at Hamaoka

Mutation scoring at Hamaoka was initiated in 1974 by Mr. Motoyuki Nagata, a biology teacher of Sagara High School, and then continued until 1977 by him and Mr. Shizuo Oki, a science teacher of Kakegawa-Nishi Middle School. They observed about 2,750,000 stamen hairs in total during the four years detecting 12,057 pink mutant events (see Table 1).

When the mutation scoring was started on July 7, 1974, the Hamaoka Nuclear Power Plant of the Chubu Electric Power Co. had a 540-MWe boiling-water-type reactor (BWR) manufactured by the General Electric Co. (GE), which was just nearing completion. The reactor was started up on August 13 of the same year for the test operation, but after increasing its power gradually up to about 300 MWe, the test operation was stopped on October 2, only 50 day after its start, because a crack was found on one of the cooling-system pipes. The resumption of the test operation had to be suspended until February 19, 1975, and the second-time test operation lasted until October 11, 1975. The regular operation of the reactor was started on March 17, 1976. The second reactor (a 840-MWe BWR of GE) was later installed in this nuclear power plant, and it reached the regular operation in November of 1978.

In 1974, the first year of observation, scoring of pink mutations in stamen hairs was made daily during the period of July 7 through October 31 (about 17 weeks) on the flowers collected from nine points around the nuclear power plant and one point (control) in Sagara, an adjacent town of Hamaoka (Fig. 2). About 640,000 stamen hairs (consisting of about 17,600,000 hair cells) in total were observed and 2,778 pink mutant events in total were detected.

When the data were grouped into ten different points times eight different periods of two weeks (the first period was of three weeks) and were subjected to a contingency chi-square test, a highly significant difference (< 0.01) in the pink mutation frequency due to point and period was found. It meant that the mutation frequency varied significantly depending on points and scoring periods. An analysis of variance also proved that the mutation frequency differed among points (< 0.01) as well as among scoring periods (< 0.01). The frequency of

pink mutations calculated was apparently higher at certain points located to the northeast to east of the nuclear plant and in the periods after August 25 and before October 5, and significantly higher mutation frequencies were found at points 1 and 5 as compared to those at Sagara in such periods. Such a tendency is seen in Table 2 in which the data from the five points (points 1 to 5 in Fig. 2) located to the northeast to east of the nuclear plant and those from the four points (points 6 to 9 in Fig. 2) located to the northwest to north are pooled separately to make the table simpler.

Simple comparison of the mutation frequency at each point in Hamaoka with that in Sagara in a corresponding period, however, may not directly help detecting a mutation increase due to radioactive release, because a difference in mutation frequency, if detected statistically, might reflect the influences of different environmental factors other than radiation levels between places. In fact, the pink mutation frequency at each point before the start of the reactor's test operation differed from each other significantly, being generally higher in Sagara than in Hamaoka, as such a tendency is seen in Table 2. The pre-operation mutation frequencies were determined based on the data collected in the period of July 7 to August 20. [The test operation was started on August 13, but not effect of a radiation exposure appears on the KU 7 stamen hairs for the first seven post-irradiation days (4).]

Therefore, comparison of the mutation data was made employing the frequency in Sagara and also that before the test operation as controls. Namely, the value obtained after subtracting the control frequency in Sagara in the same period was corrected by the difference in the pre-operation control levels of mutation frequency between Sagara and each point. The resultant values are considered to

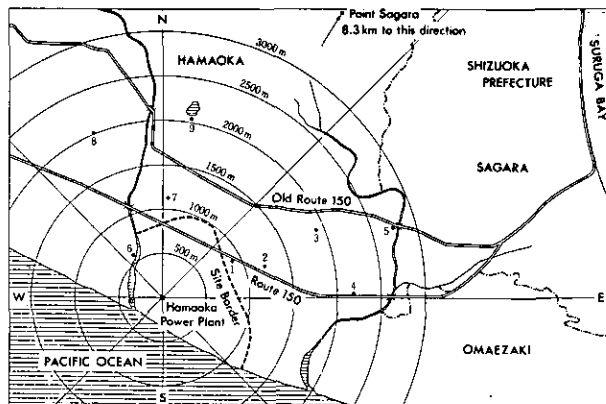


FIGURE 2. Points at which potted plants of KU 7 clone placed around the Hamaoka Nuclear Power Plant.

Table 2. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1974 around the Hamaoka Nuclear Power Plant.^a

Period	Sagara Point 10	Hamaoka			Total
		NE-E Points 1-5	NW-N Points 6-9	Subtotal	
7/7-27	4.87 ± 0.41	3.35 ± 0.36	5.02 ± 0.54	4.01 ± 0.30 ⁽⁻⁾	4.35 ± 0.25
7/28-8/10	3.35 ± 0.64	2.89 ± 0.48	2.63 ± 0.66	2.80 ± 0.39	2.97 ± 0.33
8/11-24	3.84 ± 0.43	3.76 ± 0.27	3.25 ± 0.30	3.55 ± 0.20	3.61 ± 0.18
8/25-9/7	4.13 ± 0.38	4.79 ± 0.28	4.65 ± 0.29	4.72 ± 0.20	4.61 ± 0.18
9/8-21	5.16 ± 0.50	6.54 ± 0.36 ⁺	5.13 ± 0.32	5.85 ± 0.24	5.73 ± 0.22
9/22-10/5	4.97 ± 1.08	5.06 ± 0.71	5.69 ± 0.73	5.39 ± 0.51	5.32 ± 0.46
10/6-19	3.83 ± 0.71	4.07 ± 0.43	4.45 ± 0.48	4.25 ± 0.32	4.18 ± 0.29
10/20-31	2.87 ± 0.42	2.98 ± 0.26	3.88 ± 0.33 ⁽⁺⁾	3.39 ± 0.21	3.30 ± 0.19
Total	4.21 ± 0.18	4.37 ± 0.13	4.42 ± 0.14	4.39 ± 0.09	4.36 ± 0.08
7/7-8/20	4.32 ± 0.29	3.54 ± 0.22 ⁻	3.81 ± 0.29	3.64 ± 0.17 ⁻	3.85 ± 0.15
8/25-10/14	4.54 ± 0.28	5.38 ± 0.20 ⁺⁺	4.91 ± 0.20	5.15 ± 0.14 ⁽⁺⁾	5.04 ± 0.12

^a ++ and + denote significantly higher than in Sagara at 0.02 and 0.05 levels, respectively; = and - denote significantly lower than in Sagara at 0.01 and 0.05 levels, respectively. (+) and (-) denote higher and lower than in Sagara, respectively, at the level between 0.05 and 0.10.

reflect primarily the influence(s) of some factor(s) which occurred in Hamaoka, at least additional to that in Sagara, after starting the test operation of the reactor. In Figure 3 are given those values calculated for each point in Hamaoka (those for 1975 are shown together). It is seen in this figure that in 1974 significant increases in mutation frequency occurred in the period from August 25 to October 5, especially at points 1, 5 and 6. It was also found that the value of mutation increase for the pooled data for points 1 to 5 and for the above six-week period was also statistically highly significant. The pooled mutation frequency for these five points for the period from August 25 to October 14 [12 days after starting the test operation to 12 days after stopping it; consider the maximum effect of an exposure to radiation appearing 12 days later (4, 12)] was also significantly higher than that in Sagara as seen in Table 2.

Significant increases of pink mutations were more frequently and more evidently observed in 1975 than in 1974, again more conspicuously at the points 1 to 5 located to the northeast to east of the nuclear power plant (Table 3; see also Fig. 3) and also at the point 6 closest to the reactor (see Fig. 3). A similar tendency was once again observed in 1976 (Table 4), but it was rather obscure in 1977 (Table 5).

Experiments at Shimane

Observation of the stamen hairs of KU 7 (1976) and KU 9 clones (1977 to 1979) was carried out by the Kogai (pollution) study group of students of Shimane University at Matsue. The group scored

about 490,000 and 2,350,000 stamen hairs of KU 7 and KU 9, respectively, in those four years (see Table 1).

The Shimane Nuclear Power Plant built at Kashima, Shimane, by the Chugoku Electric Power Co. has a 460-MWe BWR manufactured by the GE, and its regular operation was started in March of 1974. This means that the reactor had already been in operation when the *in situ* monitoring experiment was begun, thus no pre-operation mutation levels could be determined around this nuclear plant.

The tester *Tradescantia* plants were placed at five or six different points in Kashima and two points in Matsue (the adjacent city of Kashima and the capital of Shimane Prefecture) as shown in Figure 4. The first-year (1976) scoring of pink mutation proved highly significant increases of mutations at the point 5 located to the east of the nuclear power plant in the later half of the scoring season (August 22 to September 29), as compared with the mutation frequencies at the two points in Matsue, as seen in Table 6. The results of a contingency chi-square test and of a variance analysis were of course highly significant.

In 1977, when the largest number of KU 9 stamen hairs were observed (about 1,100,000 hairs which contained 2,613 mutant events; see Table 1), it was found that the mutation frequency in Kashima as a whole throughout the scoring period was significantly higher than that in Matsue, a busy city much more polluted in a general sense, as seen in Table 7. The frequency of mutations was almost equally high at any of the six points in Kashima regardless of the direction from the nuclear plant in this year (Table 7). Apparently higher mutation

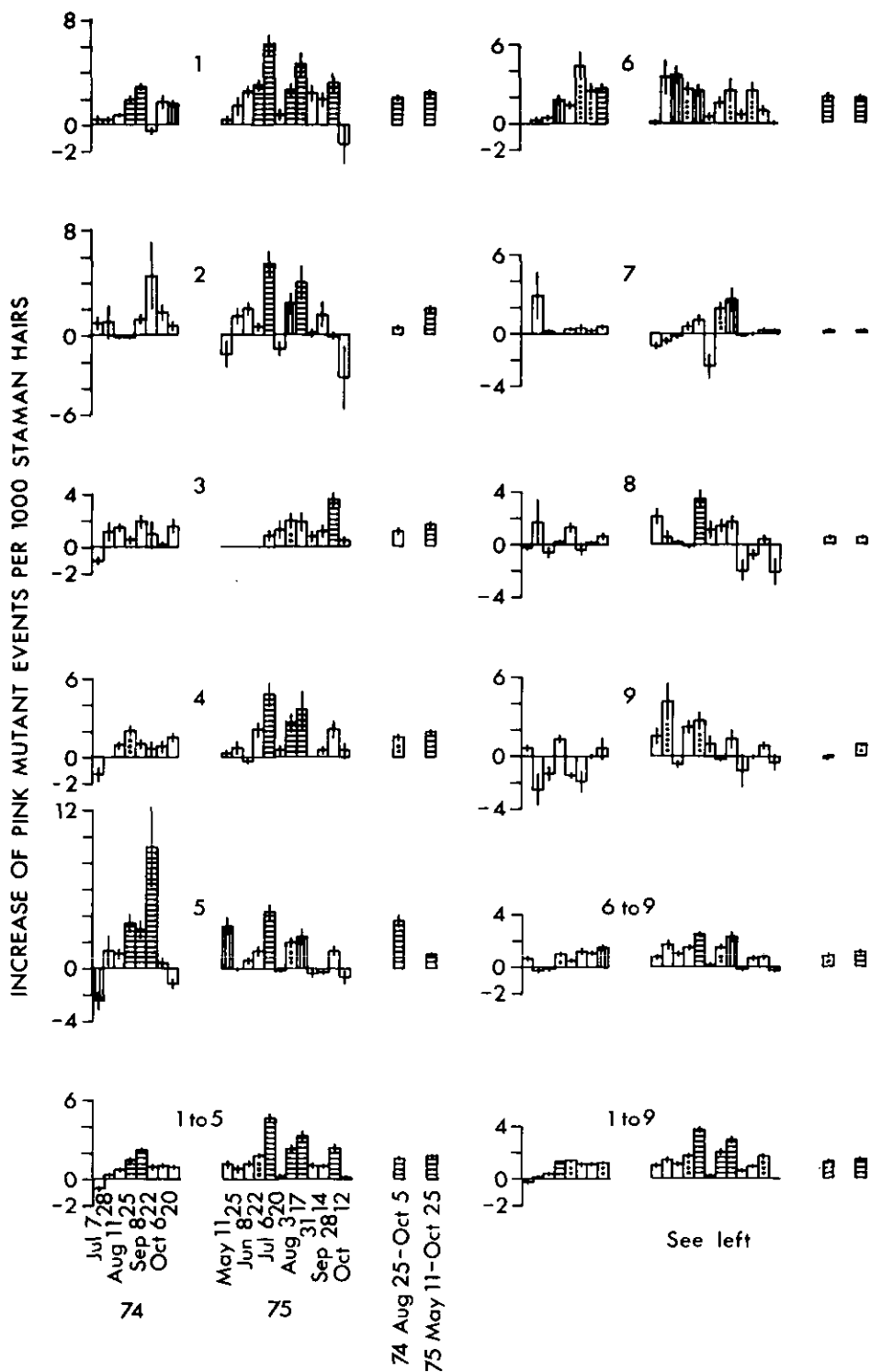


FIGURE 3. Increases of pink mutant events per 1000 stamen hairs of KU 7 clone at different points (1 to 9) near the Hamaoka Nuclear Power Plant and in different periods, calculated as explained in the text. Histograms with lateral stripes are significant at 0.01 level; those with longitudinal stripes at 0.02 level; and those dotted at 0.05 level.

Table 3. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1975 around the Hamaoka Nuclear Power Plant.^a

Period	Sagara Point 10	Hamaoka			Total
		NE-E Points 1-5	NW-N Points 6-9	Subtotal	
5/11-24	4.49 ± 0.75	4.94 ± 0.56	4.74 ± 0.46	4.82 ± 0.35	4.76 ± 0.32
5/25-6/7	5.19 ± 0.92	5.25 ± 0.76	6.39 ± 0.74	5.89 ± 0.53	5.73 ± 0.46
6/8-21	7.00 ± 0.81	7.39 ± 0.54	7.48 ± 0.54	7.43 ± 0.38	7.36 ± 0.34
6/22-7/5	5.90 ± 0.67	6.93 ± 0.45	6.92 ± 0.47	6.92 ± 0.32	6.75 ± 0.29
7/6-19	4.32 ± 0.66	8.19 ± 0.47 ⁺⁺⁺	6.30 ± 0.46 ⁺	7.836 ± 0.33 ⁺⁺⁺	6.96 ± 0.30
7/20-8/2	4.65 ± 1.04	4.08 ± 0.49	4.20 ± 0.58	4.13 ± 0.37	4.20 ± 0.35
8/3-16	2.19 ± 0.52	3.72 ± 0.32 ⁺	3.18 ± 0.36	3.50 ± 0.24 ⁽⁺⁾	3.35 ± 0.22
8/17-30	1.40 ± 0.53	3.94 ± 0.44 ⁺⁺⁺	3.21 ± 0.48 ⁺	3.64 ± 0.32 ⁺⁺	3.36 ± 0.29
8/31-9/13	3.93 ± 0.78	4.17 ± 0.47	3.34 ± 0.48	3.81 ± 0.34	3.83 ± 0.31
9/14-27	2.58 ± 0.69	2.78 ± 0.33	2.72 ± 0.37	2.76 ± 0.25	2.74 ± 0.23
9/28-10/11	3.58 ± 0.58	5.25 ± 0.41 ⁺	3.85 ± 0.37	4.58 ± 0.28	4.42 ± 0.25
10/12-25	4.17 ± 1.47	3.45 ± 0.57	3.51 ± 0.61	3.48 ± 0.42	3.54 ± 0.40
Total	4.37 ± 0.22	5.29 ± 0.14 ⁺⁺⁺	4.91 ± 0.14 ⁺	5.11 ± 0.10 ⁺⁺⁺	5.00 ± 0.09

^a + + +, ++, and + denote significantly higher than in Sagara at 0.01, 0.02, and 0.05 levels, respectively; (+) denotes higher than in Sagara at the level between 0.05 and 0.10.

Table 4. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1976 around the Hamaoka Nuclear Power Plant.^a

Period	Sagara Point 10	Hamaoka			Total
		NE-E Points 1-3,5	NW-N Points 6,9	Subtotal	
5/19-29	3.46 ± 0.55	5.95 ± 0.53 ⁺⁺⁺	5.22 ± 0.44 ⁺	5.54 ± 0.34 ⁺⁺⁺	5.14 ± 0.29
5/30-6/12	5.45 ± 0.56	5.81 ± 0.34	5.50 ± 0.41	5.69 ± 0.26	5.65 ± 0.24
6/13-26	6.39 ± 0.60	6.19 ± 0.44	5.08 ± 0.45 ⁽⁻⁾	5.71 ± 0.31	5.87 ± 0.28
6/27-7/10	7.32 ± 0.74	5.65 ± 0.41 ⁻	4.13 ± 0.44 ⁼	5.05 ± 0.30 ⁼	5.49 ± 0.29
7/11-24	4.55 ± 0.80	5.70 ± 0.80	3.53 ± 0.72	4.75 ± 0.55	4.66 ± 0.45
7/25-8/7	2.87 ± 0.64	2.55 ± 0.41	2.27 ± 0.51	2.44 ± 0.32	2.54 ± 0.29
8/8-21	3.60 ± 0.63	2.66 ± 0.30	2.47 ± 0.36 ⁽⁻⁾	2.58 ± 0.23 ⁽⁻⁾	2.75 ± 0.22
8/22-9/4	4.09 ± 0.76	2.39 ± 0.36 ⁻	2.21 ± 0.40 ⁼	2.31 ± 0.27 ⁼	2.63 ± 0.26
9/51-18	3.55 ± 0.56	3.53 ± 0.32	3.18 ± 0.44	3.42 ± 0.26	3.44 ± 0.24
9/19-10/2	4.69 ± 0.63	4.26 ± 0.29	2.90 ± 0.37 ⁼	3.86 ± 0.23	3.98 ± 0.22
10/3-16	2.93 ± 0.34	4.01 ± 0.25 ⁺⁺	3.87 ± 0.37 ⁽⁺⁾	3.97 ± 0.21 ⁺⁺	3.74 ± 0.18
10/17-31	3.19 ± 0.34	4.58 ± 0.28 ⁺⁺⁺	3.02 ± 0.34	4.08 ± 0.22 ⁺	3.86 ± 0.19
Total	4.30 ± 0.16	4.51 ± 0.10	3.89 ± 0.13 ⁻	4.28 ± 0.08	4.29 ± 0.07

^a + + +, ++, and + denotes significantly higher than in Sagara at 0.01, 0.02, and 0.05 levels, respectively; =, -, and - denote significantly lower than in Sagara at 0.01, 0.02 and 0.05 levels, respectively; (+) and (-) denote higher and lower than in Sagara, respectively, at the level between 0.05 and 0.10.

frequencies in Kashima than in Matsue were similarly observed in 1978, though the differences were less significant in general except for some clearly significant differences found at the points 1 and 5 located to the southwest and the east of the nuclear power plant, respectively (Table 8). The data collected in 1979 showed that significantly higher mutation frequencies than in Matsue were most frequently found at the points located to the south-east of the nuclear power plant rather than at the eastern point (Table 9).

Experiments at Takahama and Ohwi

The experiment was first started in 1976 around the Takahama Nuclear Power Plant and then expanded to cover the area around the Ohwi Nuclear Power Plant which was built later closely to the former (only 14 km east of the former; see Fig. 5). The mutation scoring to monitor the Takahama plant was made by Miss Akiko Kumahara, a civil servant working at the Maizuru Office of Kyoto Prefecture, observing about 400,000 KU 7

Table 5. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1977 around the Hamaoka Nuclear Power Plant.^a

Period	Sagara Points 10, 11	Hamaoka			Total
		NE-E Points 1-3,5	NW-N Points 6,9	Subtotal	
5/31-6/11	5.22 ± 0.70	5.71 ± 0.51	3.61 ± 0.54 ⁽⁻⁾	4.96 ± 0.38	5.02 ± 0.33
6/12-25	6.37 ± 0.48	7.13 ± 0.50	6.16 ± 0.61	6.78 ± 0.39	6.62 ± 0.30
6/26-7/9	4.37 ± 0.45	4.71 ± 0.40	4.47 ± 0.64	4.65 ± 0.34	4.55 ± 0.27
7/10-23	2.31 ± 0.41	3.63 ± 0.40 ⁺	2.02 ± 0.42	3.10 ± 0.30	2.87 ± 0.24
7/24-8/6	2.19 ± 0.50	3.09 ± 0.49	1.36 ± 0.55	2.65 ± 0.39	2.49 ± 0.31
8/7-20	2.94 ± 0.40	3.07 ± 0.27	2.55 ± 0.40	2.93 ± 0.22	2.93 ± 0.19
8/21-9/3	4.72 ± 0.36	3.67 ± 0.35 ⁻	3.59 ± 0.43 ⁽⁻⁾	3.64 ± 0.27 ⁼	4.09 ± 0.22
9/4-17	2.06 ± 1.03	2.89 ± 0.96	0 ± 0	2.51 ± 0.83	2.35 ± 0.65
9/18-10/1	3.28 ± 0.52	3.98 ± 0.44	1.76 ± 0.29 ⁼	2.86 ± 0.26	2.95 ± 0.23
10/2-15	3.87 ± 0.29	3.83 ± 0.23	3.88 ± 0.35	3.85 ± 0.19	3.85 ± 0.16
10/16-31	4.66 ± 0.71	3.24 ± 0.32 ⁻	2.85 ± 0.57 ⁻	3.16 ± 0.28 ⁻	3.44 ± 0.26
Total	4.20 ± 0.14	4.11 ± 0.11	3.39 ± 0.15 ⁼	3.88 ± 0.09 ⁽⁻⁾	3.98 ± 0.08

^a + denotes significantly higher than in Sagara at 0.05 levels; =, -, and - denotes significantly lower than in Sagara at 0.01, 0.02, and 0.05 levels, respectively; (-) denote lower than in Sagara, at the level between 0.05 and 0.10.

Table 6. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1976 around the Shimane Nuclear Power Plant.^a

Period	Matsue Point 7, 8	Kashima			Subtotal	Total
		E Points 5	SE Points 2-4	SW Point 1		
6/30-7/10	2.50 ± 0.43	3.39 ± 0.56	2.92 ± 0.36	2.91 ± 0.62	3.04 ± 0.27	2.91 ± 0.23
7/11-24	3.13 ± 0.57	2.40 ± 0.47	2.37 ± 0.31	2.83 ± 0.54	2.48 ± 0.24	2.59 ± 0.22
7/25-8/7	3.26 ± 0.49	2.86 ± 0.50	3.44 ± 0.34	2.84 ± 0.48	3.18 ± 0.24	3.19 ± 0.22
8/8-21	3.60 ± 0.48	3.48 ± 0.51	4.89 ± 0.42 ⁽⁺⁾	4.48 ± 0.83	4.44 ± 0.31	4.23 ± 0.26
8/22-9/4	3.86 ± 0.32	5.50 ± 0.56 ⁺⁺⁺	4.15 ± 0.34	3.09 ± 0.64	4.41 ± 0.27	4.20 ± 0.21
9/5-18	4.82 ± 0.34	6.72 ± 0.66 ⁺⁺⁺	4.77 ± 0.36	5.13 ± 0.49	5.28 ± 0.27	5.11 ± 0.21
9/19-29	2.47 ± 0.54	5.74 ± 0.74 ⁺⁺⁺	2.86 ± 0.50	3.39 ± 0.34	3.93 ± 0.34 ⁺	3.64 ± 0.29
Total	3.79 ± 0.17	4.47 ± 0.22 ⁺⁺	3.82 ± 0.14	3.72 ± 0.22	3.96 ± 0.11	3.91 ± 0.09

^a + + +, ++, and + denote significantly higher than in Matsue at 0.01, 0.02, and 0.05 levels, respectively; (+) denotes higher than in Matsue at the level between 0.05 and 0.10.

Table 7. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1977 around the Shimane Nuclear Power Plant.^a

Period	Matsue Point 7, 8	Kashima			Subtotal	Total
		E Points 5, 6	SE Points 2-4	SW Point 1		
6/12-25	2.22 ± 0.22	2.55 ± 0.25	2.44 ± 0.19	2.54 ± 0.35	2.49 ± 0.14	2.42 ± 0.12
6/26-7/9	1.82 ± 0.22	1.93 ± 0.23	2.60 ± 0.21 ⁺⁺	1.95 ± 0.32	2.28 ± 0.14 ⁽⁺⁾	2.16 ± 0.12
7/10-23	2.55 ± 0.24	2.57 ± 0.29	2.39 ± 0.22	2.30 ± 0.38	2.43 ± 0.16	2.47 ± 0.13
7/24-8/6	2.27 ± 0.23	2.51 ± 0.28	2.79 ± 0.25	3.00 ± 0.51	2.72 ± 0.17	2.57 ± 0.14
8/7-20	2.03 ± 0.26	2.83 ± 0.29 ⁺	2.14 ± 0.20	2.73 ± 0.44	2.44 ± 0.15	2.35 ± 0.13
8/21-9/3	2.54 ± 0.29	2.32 ± 0.26	2.51 ± 0.21	2.81 ± 0.41	2.50 ± 0.15	2.51 ± 0.13
9/4-17	1.72 ± 0.38	1.95 ± 0.29	2.30 ± 0.24	2.26 ± 0.35	2.19 ± 0.16	2.13 ± 0.15
9/18-10/1	1.71 ± 0.36	2.25 ± 0.42	2.01 ± 0.24	2.31 ± 0.42	2.12 ± 0.19	2.05 ± 0.17
10/2-18	0 ± 0	2.76 ± 0.37 ⁽⁺⁾	2.60 ± 0.37 ⁽⁺⁾	2.54 ± 0.38 ⁽⁺⁾	2.64 ± 0.22 ⁽⁺⁾	2.59 ± 0.21
Total	2.18 ± 0.09	2.42 ± 0.10 ⁽⁺⁾	2.42 ± 0.07 ⁺	2.46 ± 0.13 ⁽⁺⁾	2.43 ± 0.05 ⁺	2.37 ± 0.05

^a + + and + denote significantly higher than in Matsue at 0.02 and 0.05 levels, respectively; (+) denotes higher than in Matsue at the level between 0.05 and 0.10.

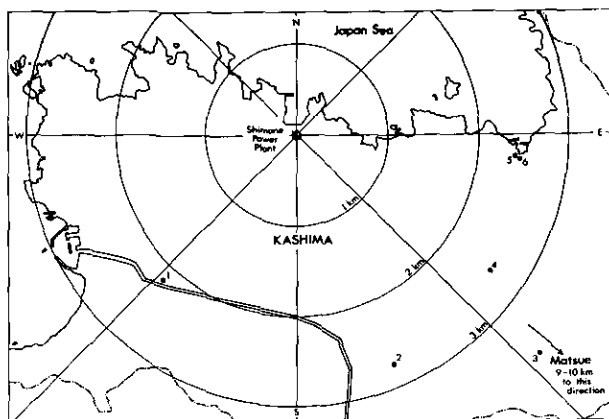


FIGURE 4. Points at which potted plants of KU 7 (1976) and KU 9 (1977 to 1979) clones placed around the Shimane Nuclear Power Plant.

stamen hairs in 1976 and as many as 4,090,000 stamen hairs of KU 9 in 1977 to 1979 (see Table 1). The *in situ* monitoring around the Ohwi plant was carried out by Miss Kiyo Nagatani, a devoted citizen working at a local clinic, who scored about 1,210,000 KU 9 stamen hairs in 1978 and 1979 (see Table 1).

Both the Takahama and Ohwi nuclear plants built at Takahama and Ohwi towns of Fukui Prefecture, respectively, are owned by the Kansai Electric Power Co. The Takahama plant is installed with two 826-MWe pressurized-water-type reactors (PWR's) manufactured by the Westinghouse Electric Corp. (WH), and the first and second reactors were started to be operated regularly in November of 1974 and November of 1975, respectively. That is, as in the case of the experiment at Shimane described above, the mutation frequency levels before the start of the reactor operation could not

be determined around this nuclear plant. In case of the Ohwi nuclear plant, on the other hand, its two huge 1,175-MWe PWR's manufactured by the WH were not yet being operated at the time (June, 1977) when the observation of *Tradescantia* stamen hairs was started, thus it was possible to determine a pre-operation mutation level. The test and regular operations of the first reactor were started in November of 1977 and March of 1979, respectively, while those of the second reactor were delayed until August of 1978 and December of 1979, respectively.

The points where the tester plants of *Tradescantia* were placed for monitoring the Takahama and Ohwi nuclear power plants are shown in Figure 5. The first-year (1976) experiment with KU 7 proved, though the scale was not very large (see Table 1), that the pink mutation frequencies in all the four points around the Takahama plant were significantly higher than that in Maizuru (11 km west-southwest of the nuclear plant), and that such significantly higher mutation frequencies were most often observed at two points located 1.2 km west and 1.3 km south of the nuclear plant (Table 10).

More intensive scoring was made in 1977, with observation of as many as about 1,320,000 stamen hairs of KU 9 clone and detection of nearly 4,000 pink mutant events (see Table 1). Highly significant differences in mutation frequency were clearly observed between Maizuru (two points) and three points in Takahama, the mutation frequency being higher at the latter as shown in Table 11. Especially, the frequencies of pink mutations at the points 1.3 km south of the nuclear plant were often higher significantly than those in Maizuru as seen in the same table (mutation scoring at the point 1.2 km west of the nuclear plant could not be made in this year).

In this second year of the experiment, mutation

Table 8. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1978 around the Shimane Nuclear Power Plant.^a

Period	Matsue Points 7, 8	Kashima			Subtotal	Total
		E Points 5	SE Points 2-4	SW Point 1		
5/21-6/3	1.95 ± 0.22	2.25 ± 0.31	2.10 ± 0.18	2.84 ± 0.34 ⁺⁺	2.29 ± 0.14	2.20 ± 0.12
6/4-17	1.78 ± 0.24	2.05 ± 0.32	1.92 ± 0.18	2.29 ± 0.35	2.02 ± 0.14	1.96 ± 0.12
6/18-7/1	1.42 ± 0.20	1.74 ± 0.31	1.94 ± 0.19 ⁽⁺⁾	1.55 ± 0.32	1.83 ± 0.15	1.71 ± 0.12
7/2-15	1.22 ± 0.22	2.22 ± 0.41 ⁺⁺	1.27 ± 0.17	1.37 ± 0.32	1.47 ± 0.15	1.40 ± 0.12
7/16-29	1.31 ± 0.22	1.01 ± 0.30	1.36 ± 0.22	1.30 ± 0.34	1.27 ± 0.16	1.29 ± 0.13
7/30-8/12	2.05 ± 0.34	2.07 ± 0.55	1.22 ± 0.31 ⁽⁻⁾	1.55 ± 0.39	1.53 ± 0.23	1.72 ± 0.19
8/13-26	2.58 ± 0.86	0.81 ± 0.40 ⁻	1.45 ± 0.33	1.95 ± 0.65	1.41 ± 0.25	1.57 ± 0.24
8/27-9/6		3.64 ± 3.63	2.24 ± 1.12	2.38 ± 1.19	2.41 ± 0.80	2.41 ± 0.80
Total	1.65 ± 0.10	1.89 ± 0.14	1.76 ± 0.08	1.99 ± 0.14 ⁺	1.83 ± 0.06	1.87 ± 0.05

^a ++ and + denote significantly higher than in Matsue at 0.02 and 0.05 levels, respectively; - denotes significantly lower than in Matsue at 0.05 level; (+) and (-) denote higher and lower than in Matsue, respectively, at the level between 0.05 and 0.10.

Table 9. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1979 around the Shimane Nuclear Power Plant.^a

Period	Matsue Points 7, 8	Kashima				Subtotal	Total
		E Point 5	SE Points 2-4	SW Point 1			
6/10-23	2.43 ± 0.27	1.96 ± 0.33	2.68 ± 0.25	1.84 ± 0.32	2.32 ± 0.17	2.35 ± 0.14	
6/24-7/7	1.44 ± 0.20	2.26 ± 0.29 ⁺ ⁺	2.33 ± 0.25 ⁺ ⁺ ⁺	1.97 ± 0.35	2.23 ± 0.17 ⁺ ⁺ ⁺	1.98 ± 0.13	
7/8-21	2.48 ± 0.27	2.28 ± 0.36	2.53 ± 0.25	2.53 ± 0.43	2.47 ± 0.18	2.47 ± 0.15	
7/22-8/4	1.49 ± 0.32	0.94 ± 0.36	1.65 ± 0.32	1.53 ± 0.54	1.44 ± 0.23	1.46 ± 0.18	
8/5-18	2.93 ± 0.31	1.43 ± 0.30 ⁼	3.95 ± 0.46 ⁽⁺⁾	3.88 ± 0.71	2.99 ± 0.26	2.96 ± 0.20	
8/19-9/1	2.53 ± 0.37	1.14 ± 0.34 ⁼	1.81 ± 0.36	1.85 ± 1.07	1.56 ± 0.25 ⁻	1.97 ± 0.21	
9/2-15	1.69 ± 0.34	1.77 ± 0.56	3.71 ± 0.59 ⁺ ⁺ ⁺		3.03 ± 0.43 ⁺ ⁺	2.40 ± 0.28	
9/16-29	1.23 ± 0.36	2.62 ± 2.62	2.85 ± 0.37 ⁺ ⁺ ⁺		2.84 ± 0.36 ⁺ ⁺ ⁺	2.34 ± 0.27	
9/30-10/15	2.14 ± 0.42		2.84 ± 0.33		2.84 ± 0.33	2.62 ± 0.26	
Total	2.15 ± 0.10	1.85 ± 0.13 ⁽⁻⁾	2.66 ± 0.11 ⁺ ⁺ ⁺	2.25 ± 0.19	2.38 ± 0.08 ⁽⁺⁾	2.30 ± 0.06	

^a ++ and + denote significantly higher than in Matsue at 0.01 and 0.02 levels, respectively; =, -, and - denote significantly lower than in Matsue at 0.01, 0.02, and 0.05 levels, respectively; (+) and (-) denote higher and lower than in Matsue, respectively, at the level between 0.05 and 0.10.

Table 10. Numbers of pink mutant events per 1000 stamen hairs of KU 7 clone observed in 1976 around the Takahama Nuclear Power Plant.^a

Period	Maizuru Point 8	Takahama			Subtotal	Total
		N Points 1	S-W Points 2, 4	NW Point 6		
7/5-10	4.14 ± 1.31	4.41 ± 0.75	5.32 ± 0.64	3.37 ± 0.93	4.73 ± 0.44	4.67 ± 0.42
7/11-24	3.65 ± 0.64	3.36 ± 0.65	6.10 ± 0.61 ⁺⁺	5.17 ± 0.86	5.19 ± 0.41 ⁽⁺⁾	4.86 ± 0.35
7/25-8/7	3.15 ± 0.63	3.32 ± 0.92	5.05 ± 0.91 ⁽⁺⁾	3.49 ± 0.78	4.06 ± 0.51	3.75 ± 0.40
8/8-21	2.87 ± 0.40	3.54 ± 0.60	3.51 ± 0.38	2.50 ± 0.57	3.33 ± 0.28	3.22 ± 0.24
8/22-9/4	1.86 ± 0.38	3.94 ± 0.61 ⁺⁺⁺	3.18 ± 0.39 ⁺	4.93 ± 0.66 ⁺⁺⁺	3.82 ± 0.30 ⁺⁺⁺	3.37 ± 0.25
9/5-18	4.56 ± 0.54	5.90 ± 0.63	6.20 ± 0.62 ⁺	6.33 ± 0.75 ⁺	6.13 ± 0.38 ⁺	5.70 ± 0.31
9/19-10/2	4.57 ± 0.57	5.40 ± 0.74	5.41 ± 0.53	4.70 ± 0.61	5.19 ± 0.35	5.03 ± 0.30
10/3-16	5.42 ± 0.68	6.20 ± 0.90	6.43 ± 0.58	5.74 ± 0.65	6.15 ± 0.39	5.98 ± 0.34
10/17-23	4.85 ± 0.87	6.43 ± 1.85	4.79 ± 0.59	7.47 ± 0.85 ⁺	5.98 ± 0.48	5.76 ± 0.42
Total	3.86 ± 0.20	4.72 ± 0.25 ⁺⁺⁺	5.00 ± 0.18 ⁺⁺⁺	5.15 ± 0.25 ⁺⁺⁺	4.97 ± 0.13 ⁺⁺⁺	4.71 ± 0.11

^a + + +, + +, and + denote significantly higher than in Maizuru at 0.01, 0.02, and 0.05 levels, respectively; (+) denotes higher than in Maizuru at the level between 0.05 and 0.10.

Table 11. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1977 around the Takahama Nuclear Power Plant.^a

Period	Maizuru Point 8, 9	Takahama				Ohwi (Hongo) Point 12	Total
		N Point 1	S Point 2	NW Point 6	Subtotal		
5/9-22	6.45 ± 1.29	6.34 ± 0.68	3.19 ± 0.80 ⁻	6.14 ± 0.73	5.74 ± 0.44		5.82 ± 0.41
5/23-6/5	4.10 ± 0.31	5.93 ± 0.41 ⁺⁺⁺	5.06 ± 0.77	4.27 ± 0.40	5.19 ± 0.27 ⁺⁺⁺		4.78 ± 0.20
6/6-19	3.92 ± 0.24	4.09 ± 0.35	3.79 ± 0.34	4.60 ± 0.38	4.15 ± 0.21		4.06 ± 0.16
6/20-7/3	3.13 ± 0.22	3.25 ± 0.32	3.78 ± 0.36	3.10 ± 0.31	3.36 ± 0.19	2.72 ± 0.42	3.22 ± 0.14
7/4-17	2.33 ± 0.21	3.12 ± 0.35 ⁺	3.68 ± 0.41 ⁺⁺	3.50 ± 0.46 ⁺⁺	3.41 ± 0.23 ⁺⁺⁺	2.28 ± 0.30	2.81 ± 0.14
7/18-31	2.11 ± 0.22	1.97 ± 0.34	3.12 ± 0.45 ⁺	2.15 ± 0.35	2.38 ± 0.22	1.88 ± 0.28	2.18 ± 0.14
8/1-14	2.06 ± 0.22	2.50 ± 0.36	5.25 ± 0.68 ⁺⁺⁺	3.05 ± 0.48 ⁺	3.38 ± 0.28 ⁺⁺⁺	2.45 ± 0.32	2.66 ± 0.15
8/15-28	2.30 ± 0.20	2.28 ± 0.30	2.76 ± 0.44	2.69 ± 0.35	2.53 ± 0.20	1.73 ± 0.24 ⁽⁻⁾	2.28 ± 0.12
8/29-9/11	2.24 ± 0.20	2.04 ± 0.29	2.85 ± 0.38	2.49 ± 0.33	2.43 ± 0.19	2.62 ± 0.29	2.40 ± 0.13
9/12-25	1.92 ± 0.20	2.46 ± 0.43	3.74 ± 0.80 ⁺⁺⁺	2.43 ± 0.35	2.64 ± 0.26 ⁺	2.30 ± 0.31	2.25 ± 0.14
9/26-10/1	2.55 ± 0.27	2.22 ± 1.57	2.80 ± 1.61	2.10 ± 0.74	2.25 ± 0.62	1.96 ± 0.38	2.37 ± 0.21
Total	2.74 ± 0.07	3.51 ± 0.12 ⁺⁺⁺	3.65 ± 0.15 ⁺⁺⁺	3.39 ± 0.13 ⁺⁺⁺	3.51 ± 0.08 ⁺⁺⁺	2.23 ± 0.11*	3.03 ± 0.05

^a + + +, + +, and + denote significantly higher than in Maizuru at 0.01, 0.02, and 0.05 levels, respectively; (-) denotes lower than in Maizuru at the level between 0.05 and 0.10; * denotes not significantly different from 6/20-10/1 data from Maizuru.

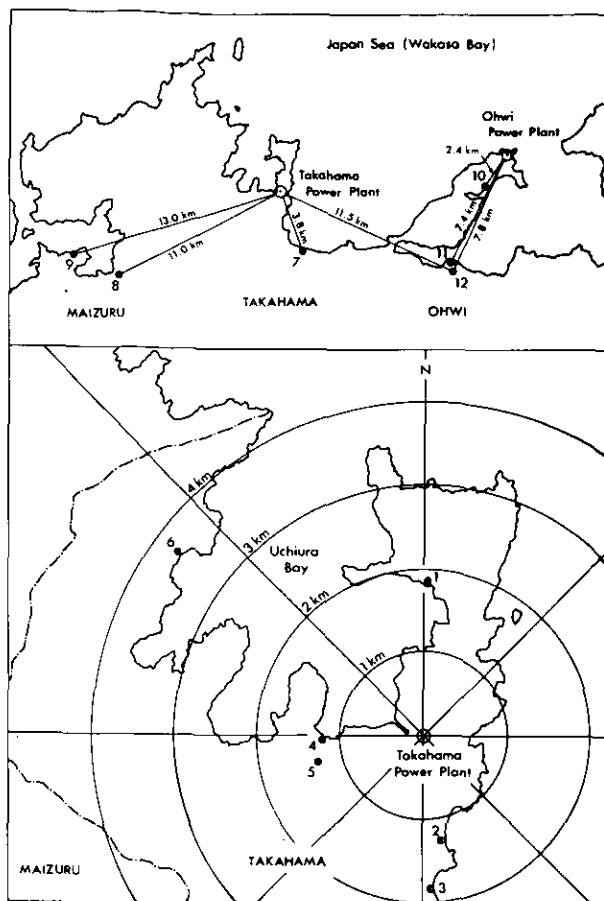


FIGURE 5. Points at which potted plants of KU 7 (1976) and KU 9 (1977 to 1979) clones placed around the Takahama and Ohwi nuclear power plants.

scoring was initiated also at one point in Ohwi town in order to determine the mutation frequency level prior to the start of operation of the Ohwi plant. The data obtained showed no statistical difference from those taken in Maizuru, indicating the mutation frequency at Ohwi in this year was essentially identical to that in Maizuru (Table 11).

Very similar results were obtained in 1978 after conducting scorings on the largest sample size of about 1,570,000 stamen hairs (see Table 1). Significantly increased mutation frequencies were observed at any of the five points in Takahama investigated, especially conspicuously at the two points located 1.3 km west and 1.3 km south of the nuclear plant as seen in Table 12. The mutation frequency at Ohwi (Hongo) was not different statistically from that in Maizuru throughout the scoring season (Table 12), proving that the frequency at this point can be used as the control for the experiment around the Ohwi nuclear plant.

No statistical difference at all was found in the mutation data collected in 1979 (Table 13), although a considerably large sample size was examined (about 1,200,000 stamen hairs; see Table 1). It should be noted that both the first and the second reactors of the Takahama plant were being stopped throughout the scoring periods of this year, because serious troubles had occurred in both reactors.

The data taken around the Ohwi nuclear power plant in 1978 are summarized in Table 14. The mutation frequencies at the point in Oshima 2.4 km south-southwest of the nuclear plant were often significantly higher than those at the two points in Hongo (7.4 and 7.8 km south-southwest). The

Table 12. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1978 around the Takahama Nuclear Power Plant.^a

Period	Takahama						Ohwi (Hongo) Point 12	Total
	Maizuru Points 8, 9	N Point 1	SSE Point 7	S-W Points 2, 5	NW Point 6	Subtotal		
5/1-14	3.41 ± 0.24	3.27 ± 0.34	3.54 ± 0.39	3.84 ± 0.28	3.79 ± 0.45	3.64 ± 0.18	3.03 ± 0.35	3.50 ± 0.13
5/15-28	3.41 ± 0.22	3.38 ± 0.37	3.05 ± 0.37	3.69 ± 0.26	3.91 ± 0.38	3.56 ± 0.17	3.04 ± 0.30	3.44 ± 0.12
5/29-6/11	3.07 ± 0.24	3.31 ± 0.36	3.01 ± 0.34	3.63 ± 0.27	2.82 ± 0.41	3.31 ± 0.17	2.72 ± 0.33	3.17 ± 0.13
6/12-25	2.11 ± 0.21	2.97 ± 0.35 ⁺	2.71 ± 0.41	3.06 ± 0.25 ⁺⁺	3.09 ± 0.38 ⁺⁺	3.00 ± 0.17 ⁺⁺⁺	2.52 ± 0.35	2.70 ± 0.12
6/26-7/9	1.68 ± 0.19	1.61 ± 0.28	1.73 ± 0.30	2.05 ± 0.22	2.10 ± 0.34	1.90 ± 0.14	1.56 ± 0.26	1.80 ± 0.10
7/10-23	1.27 ± 0.20	1.81 ± 0.32	2.27 ± 0.42 ⁺⁺	2.14 ± 0.26 ⁺⁺⁺	1.91 ± 0.34 ⁽⁺⁾	2.04 ± 0.16 ⁺⁺⁺	1.68 ± 0.31	1.79 ± 0.12
7/24-8/6	1.43 ± 0.22	1.76 ± 0.34	2.11 ± 0.40	2.55 ± 0.31 ⁺⁺⁺	1.96 ± 0.45	2.19 ± 0.18 ⁺⁺	1.91 ± 0.32	1.95 ± 0.13
8/7-20	1.63 ± 0.21	2.20 ± 0.41	1.90 ± 0.38	2.25 ± 0.32 ⁽⁺⁾	1.89 ± 0.43	2.09 ± 0.19	1.82 ± 0.33	1.90 ± 0.13
8/21-9/3	1.25 ± 0.19	0.96 ± 0.29	1.48 ± 0.30	2.04 ± 0.31 ⁺	1.87 ± 0.44	1.64 ± 0.17	1.44 ± 0.30	1.49 ± 0.12
9/4-17	1.95 ± 0.23	2.62 ± 0.55	1.97 ± 0.36	1.80 ± 0.28	1.51 ± 0.76	1.98 ± 0.20	2.20 ± 0.53	1.99 ± 0.15
9/18-10/1	2.51 ± 0.30		1.94 ± 0.69	2.38 ± 0.47		2.26 ± 0.39		2.42 ± 0.24
Total	2.33 ± 0.07	2.57 ± 0.12 ⁽⁺⁾	2.47 ± 0.12	2.89 ± 0.09 ⁺⁺⁺	2.79 ± 0.14 ⁺⁺⁺	2.72 ± 0.05 ⁺⁺⁺	2.29 ± 0.11	2.55 ± 0.04

^a ++, +, and + denote significantly higher than in Maizuru at 0.01, 0.02, and 0.05 levels, respectively; (+) denotes higher than in Maizuru at the level between 0.05 and 0.10.

Table 13. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1979 around the Takahama Nuclear Power Plant.

Period	Takahama				Total
	Maizuru Points 8, 9	N Point 1	S-W Points 3, 5	Subtotal	
5/21-27	5.75 ± 0.44	4.96 ± 0.58	5.99 ± 0.48	5.62 ± 0.37	5.67 ± 0.28
5/28-6/10	4.61 ± 0.27	4.32 ± 0.39	4.25 ± 0.26	4.27 ± 0.22	4.41 ± 0.17
6/11-24	2.41 ± 0.20	2.35 ± 0.27	2.66 ± 0.21	2.55 ± 0.16	2.49 ± 0.13
6/25-7/8	1.61 ± 0.18	1.57 ± 0.26	1.40 ± 0.16	1.45 ± 0.14	1.51 ± 0.11
7/9-22	1.54 ± 0.18	1.41 ± 0.24	1.65 ± 0.18	1.57 ± 0.15	1.56 ± 0.11
7/23-8/5	1.00 ± 0.15	1.30 ± 0.25	1.10 ± 0.16	1.17 ± 0.13	1.10 ± 0.10
8/6-19	1.11 ± 0.17	1.38 ± 0.26	1.41 ± 0.19	1.40 ± 0.15	1.28 ± 0.11
8/20-9/2	1.11 ± 0.17	0.90 ± 0.21	1.21 ± 0.18	1.09 ± 0.14	1.10 ± 0.11
9/3-16	0.99 ± 0.14	1.22 ± 0.22	1.32 ± 0.18	1.28 ± 0.14	1.16 ± 0.10
9/17-30	1.10 ± 0.13	1.10 ± 0.17	1.11 ± 0.14	1.10 ± 0.11	1.10 ± 0.08
Total	2.10 ± 0.07	1.95 ± 0.09	2.15 ± 0.07	2.08 ± 0.05	2.09 ± 0.04

Table 14. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1978 around the Ohwi Nuclear Power Plant.^a

Period	Ohwi (Hongo)		Total
	Points 1, 2	Ohwi (Oshima) SSW Point 10	
5/1-14	3.52 ± 0.26	3.54 ± 0.48	3.52 ± 0.23
5/15-28	3.29 ± 0.22	4.02 ± 0.36 ⁽⁺⁾	3.51 ± 0.19
5/29-6/11	3.13 ± 0.22	4.63 ± 0.38 ⁺⁺⁺	3.62 ± 0.19
6/12-25	2.83 ± 0.23	4.52 ± 0.40 ⁺⁺⁺	3.41 ± 0.21
6/26-7/9	1.95 ± 0.19	2.65 ± 0.35 ⁽⁺⁾	2.15 ± 0.17
7/10-23	2.01 ± 0.21	1.94 ± 0.33	1.99 ± 0.18
7/24-8/6	2.10 ± 0.24	1.58 ± 0.34	1.96 ± 0.20
8/7-20	1.63 ± 0.22	2.31 ± 0.43	1.81 ± 0.20
8/21-9/3	1.93 ± 0.31	1.02 ± 0.27 ⁻	1.55 ± 0.21
9/4-8	1.03 ± 0.46	1.63 ± 0.81	1.23 ± 0.41
Total	2.62 ± 0.08	3.26 ± 0.13 ⁺⁺⁺	2.81 ± 0.07

^a+++ denotes significantly higher than in Hongo at 0.01 level; - denotes significantly lower than in Hongo at 0.05 level; (+) denotes higher than in Hongo at the level between 0.05 and 0.10.

average frequency for the whole scoring season was also significantly higher at Oshima than at Hongo as seen in the table.

Pink mutations were detected at almost comparable frequencies at both Hongo and Oshima in 1979 (Table 15), being different from the results in 1978. However, the frequencies of mutations were evidently higher in general as compared with those observed in Takahama in the same year (see Table 13). Also, the average frequencies for the whole scoring period at both Hongo and Oshima were considerably higher than that at Hongo in 1978 and were comparable to that at Oshima in the preceding year (see Table 14). Conspicuous in the data presented in Table 15 is that the frequencies at Oshima and also Hongo in the period of August 6 to 19 are

very high, showing sudden jumps which are hardly expected in the high-temperature period (see Discussion).

Experiments at Tokai

The *in situ* monitoring experiments around the nuclear power complex in Tokai were carried out by Mr. Yasubumi Ueda, a teacher of Johoku High School, being assisted by Miss Minako Arakawa, Mrs. Kazuko Ueda, and other citizens including a physicist. They made mutation scorings on about 710,000 stamen hairs of KU 9 clone in 1978 and 1979 (see Table 1), a rather smaller sample size as compared to the above-described experiments at other places, but they succeeded in detecting some significant increases of mutations.

In Tokai, there are many nuclear facilities forming a huge nuclear power complex: the Nuclear Fuel Reprocessing Facility of the Japan Power Reactor and Nuclear Fuel Development Corp., the Tokai Nuclear Power Plant with a 166-MWe gas-cooled-type reactor (GCR) and the Tokai Second Nuclear Power Plant with a 1,100-MWe BWR of the Japan Atomic Power Co., the Japan Atomic Energy Research Institute with several research reactors, and some other nuclear facilities of private companies (see Fig. 6). Therefore, the *in situ* monitoring experiments with *Tradescantia* had to cover a wide area in order to detect the effects of the nuclear power complex, thus the tester plants were placed at one point in Hitachi, nine points in Tokai, one point in Katsuta, and one point in Mito (control) as shown in Figure 6.

The frequencies of pink mutations detected in the 1978 experiment were considerably lower in general as compared to those determined in other experiments described above (see Table 1). The

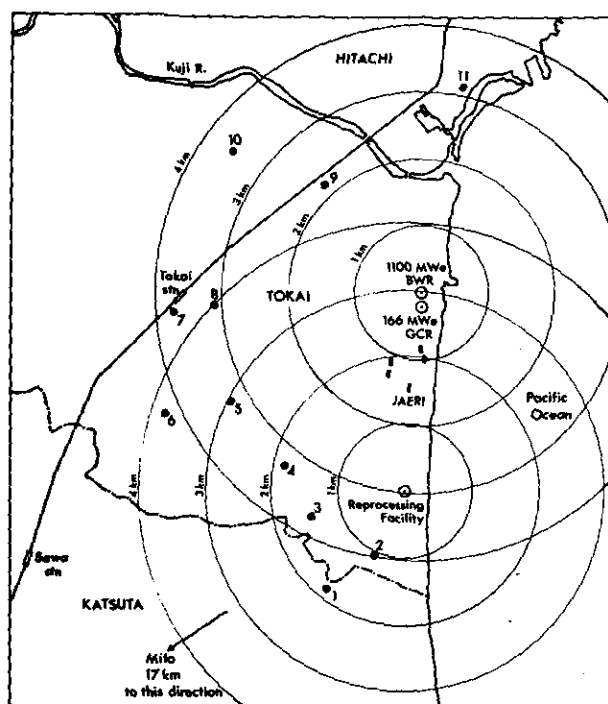


FIGURE 6. Points at which potted plants of KU 9 clone placed around the nuclear power complex in Tokai.

main reason for the discrepancy was considered to be that the light source they used for the stamen-hair observation in this year was not of the most appropriate type especially to get a clear contrast between normal blue and mutant pink colors of hair cells. Nevertheless, significantly higher mutation frequencies were detected at the three points (point 1 to 3) located to the southwest of the nuclear fuel reprocessing facility and at the three points (points 4, 5 and 7) located to the west of the nuclear power complex (Table 16). Average mutation frequency

Table 15. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1979 around the Ohwi Nuclear Power Plant.^a

Period	Ohwi (Hongo) Points 1, 2	Ohwi (Oshima) SSW Point 10	Total
5/21-27	5.91 ± 0.44	6.12 ± 0.55	5.99 ± 0.34
5/28-6/10	5.74 ± 0.30	5.39 ± 0.40	5.62 ± 0.24
6/11-24	3.21 ± 0.22	2.04 ± 0.26	2.85 ± 0.17
6/25-7/8	2.40 ± 0.21	2.15 ± 0.28	2.31 ± 0.17
7/9-22	2.19 ± 0.22	2.28 ± 0.32	2.22 ± 0.18
7/23-8/5	1.36 ± 0.20	2.13 ± 0.35 ⁺	1.62 ± 0.18
8/6-19	2.55 ± 0.29	2.75 ± 0.43	2.61 ± 0.24
8/20-9/2	1.73 ± 0.21	1.83 ± 0.33	1.76 ± 0.18
9/3-14	2.38 ± 0.26	2.22 ± 0.52	2.35 ± 0.23
Total	3.17 ± 0.09	3.17 ± 0.13	3.17 ± 0.07

^a + denotes significantly higher than in Hongo at 0.05 level; = denotes significantly lower than in Hongo at 0.01 level.

for the eight points in Tokai and Katsuta throughout the scoring period was also significantly higher than that at Mito, Table 16.

In 1979, significant differences in mutation frequency were much less frequently detected, but the average frequency for the five points (points 4 to 8) throughout the scoring period and also that for all the nine points in Tokai and Katsuta were significantly higher than that at Mito (Table 17).

Discussion

Higher Mutation Frequencies Occurred around Nuclear Plants

Highly significantly increased pink mutation frequencies in the stamen hairs of KU 7 and KU 9

Table 16. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1978 around the nuclear power complex in Tokai.^a

Period	Tokai-Katsuta					Ohwi Hitachi Point 11	Total
	Mito Point 12	SW Points 1-3	W Points 4,5,7	NW Points 9,10	Subtotal		
6/12-25	1.07 ± 0.40	1.40 ± 0.32	1.34 ± 0.45	0.62 ± 0.22	1.08 ± 0.18	1.10 ± 0.42	1.08 ± 0.15
6/26-7/9	0 ± 0	1.59 ± 0.36 ⁽⁺⁾	0.43 ± 0.25	0.89 ± 0.26	1.06 ± 0.18	0.90 ± 0.52	0.99 ± 0.16
7/10-23	0.68 ± 0.30	1.12 ± 0.35	1.19 ± 0.42	0.98 ± 0.28	1.08 ± 0.20	0.43 ± 0.43	0.96 ± 0.16
7/24-8/6	0.35 ± 0.25	2.14 ± 0.59 ⁺⁺⁺	0.36 ± 0.25	0.93 ± 0.31	1.12 ± 0.23 ⁽⁺⁾	0.48 ± 0.28	0.87 ± 0.16
8/7-20	0.85 ± 0.35	1.05 ± 0.34	3.19 ± 0.80 ⁺⁺	1.58 ± 0.29	1.71 ± 0.23 ⁽⁺⁾	1.36 ± 0.43	1.52 ± 0.18
8/21-9/3	0.83 ± 0.48	1.57 ± 0.42	1.45 ± 0.46	0.68 ± 0.20	1.07 ± 0.18	0.90 ± 0.37	1.03 ± 0.15
9/4-17	0 ± 0	1.20 ± 0.85	1.23 ± 0.50	1.09 ± 0.34	1.15 ± 0.27		1.11 ± 0.26
Total	0.70 ± 0.15	1.45 ± 0.16 ⁺⁺⁺	1.26 ± 0.17 ⁺⁺	0.99 ± 0.10	1.19 ± 0.08 ⁺⁺⁺	0.93 ± 0.17	1.09 ± 0.06

^a +++ and ++ denote significantly higher than in Mito at 0.01 and 0.02 levels, respectively; (+) denotes higher than in Mito at the level between 0.05 and 0.10.

Table 17. Numbers of pink mutant events per 1000 stamen hairs of KU 9 clone observed in 1979 around the nuclear power complex in Tokai.^a

Period	Mito Point 12	Tokai-Katsuta				Total
		SW Points 1,3	W Points 4-8	NW Points 9,10	Subtotal	
5/23-6/5	1.92 ± 0.45	1.97 ± 0.30	2.80 ± 0.27	2.39 ± 0.36	2.48 ± 0.18	2.42 ± 0.17
6/6-19	1.79 ± 0.54	1.53 ± 0.30	2.95 ± 0.29	1.97 ± 0.37	2.37 ± 0.19	2.32 ± 0.18
6/20-7/3	0.98 ± 0.57	0.83 ± 0.34	2.09 ± 2.08	1.13 ± 0.38	1.68 ± 0.20	1.63 ± 0.19
7/4-17	1.76 ± 0.66	1.83 ± 0.42	1.90 ± 0.34	1.04 ± 0.39	1.71 ± 0.22	1.71 ± 0.21
7/18-31		2.18 ± 0.63	1.82 ± 0.49	2.10 ± 1.21	1.98 ± 0.37	1.98 ± 0.37
8/1-14	0 ± 0	1.25 ± 0.27	1.22 ± 0.21	0.85 ± 0.85	1.22 ± 0.16	1.20 ± 0.16
8/15-28	1.56 ± 0.43	2.27 ± 0.47	1.14 ± 0.24		1.53 ± 0.23	1.54 ± 0.20
8/29-9/11	0.53 ± 0.38	2.33 ± 0.64 ⁺	2.10 ± 0.49 ⁺	0.61 ± 0.35	1.78 ± 0.30 ⁽⁺⁾	1.58 ± 0.26
9/12-25	1.30 ± 0.53	1.24 ± 0.41	2.06 ± 0.36	0.68 ± 0.30	1.52 ± 0.22	1.49 ± 0.21
9/26-10/9	0.92 ± 0.46	1.61 ± 0.43	2.11 ± 0.37	2.42 ± 1.21	1.96 ± 0.28	1.81 ± 0.25
10/10-20	1.25 ± 0.72	1.29 ± 0.53	0.78 ± 0.26	1.26 ± 0.56	0.99 ± 0.22	1.02 ± 0.21
Total	1.43 ± 0.17	1.67 ± 0.12	2.06 ± 0.10 ⁺⁺⁺	1.61 ± 0.15	1.87 ± 0.07 ⁺	1.83 ± 0.06

^a ++ and + denote significantly higher than in Mito at 0.01 and 0.05 levels, respectively; (+) denotes higher than in Mito at the level between 0.05 and 0.10.

clones of *Tradescantia* have been repeatedly found in the long-term *in situ* monitoring experiments at large scales (with ca. 12 million hairs observed) performed around the nuclear power plants at Hamaoka, Shimane, Takahama, Ohwi, and Tokai (also around the spent nuclear fuel reprocessing facility at Tokai). The data obtained in those experiments are summarized in Tables 2-5 (Hamaoka, 1974 to 1977), 6-9 (Shimane, 1976 to 1979), 10-13 (Takahama, 1976 to 1979), 14 and 15 (Ohwi, 1978 and 1979), and 16 and 17 (Tokai, 1978 and 1979). Most of the data are reported for the first time, except for some results from Hamaoka reported earlier by ourselves (20, 21) or introduced by Grossman (22).

The significantly increased incidence of mutations was hardly regarded to have occurred at random. The increases in the mutation frequency examined to be significant as compared to the frequencies at the control point(s) in the corresponding periods tended to be found repeatedly at particular points near nuclear plants. In Hamaoka and Ohwi (Hongo) where the mutation frequencies before the operation of the nuclear reactors could be determined, significantly higher mutation frequencies than such pre-operation levels were also found at such particular points more frequently than at other points. Also, such significantly higher mutation frequencies tended to occur in some particular periods.

Such occurrences of significantly increased mutations not at random suggest strongly the existence of some mutagenic factor(s) in the environment near nuclear facilities, which distributed unequally at different points and at different times.

Correlations of Mutation Data with Environmental Factors

For identifying such mutagenic factor(s) which caused mutation increases, the nature of the *Tradescantia* stamen-hair test system to be taken into consideration. The genetic effect of an acute radiation exposure first appears seven and eight days later in the stamen hairs of KU 9 and KU 7 clones, respectively, and the maximum effect after 12 days (4, 5). In case of a continuous chronic exposure, the maximum effect can be observed about three weeks after starting irradiation (see Fig. 7). These time intervals are of course modified by temperature, being longer at lower temperature. With such nature of the test system in mind, it is reasonable to assume that the mutagenic factor(s) must have existed at least seven or eight days before the detection of a mutation increase, most probably 12 days (in case of a single acute exposure to mutagen) or three weeks (in case of a continuous chronic exposure to mutagen) before the detection.

Possible mutagenic factors considered to have been in existence in the environment are pesticides (fungicides, insecticides and herbicides), some automotive air pollutants (NO_x, SO₂, etc.), industrial pollutants, radioactive fallout due to nuclear explosion tests, and natural radioactivity, besides radioactive nuclides released from nuclear plants. Wind and rainfall might have affected the intensities of those factors. Temperature is also known to modify spontaneous mutation frequency in *Tradescantia* stamen hairs (18, 23).

As for pesticides, special care had been taken to prevent the tester *Tradescantia* plants from being

exposed to pesticides by asking the inhabitants (mostly farmers) around any points of the monitoring experiments not to use such chemicals near the tester plants. We had also asked them to record any uses of pesticides when the uses were unavoidable, since some of the tester plants were placed near paddy or crop fields (though most of them were placed in the yards or gardens of private houses), and the data which might have been influenced by the uses were excluded if judged to be necessary. Any other information of using pesticides in the surrounding areas was also collected as much as possible with the helps of scorers, but we could not find any evidences of concentrated uses only at the points and in the periods where and when mutation increase were observed, in any of the experiments reported here.

The amount of automotive air pollutants must have been proportional to the traffic in the area. Any of the areas surrounding nuclear power plants were, however, those places where much less traffic was seen as compared to the control points which were mostly set in adjacent cities. There was considerable traffic at some points in Tokai, but far less than in the area near the control point in Mito city.

Essentially no polluting industrial plants (other than nuclear plants) exist in any of the areas surrounding nuclear plants excepting Hitachi-Tokai-Katsuta area where some nonnuclear industrial factories are operated. This is just reflecting the situation that those towns accepted the construction of nuclear plants only because they had no other industries benefiting them with economical development. Compared to those areas, the control points are much more polluted with a variety of polluting sources. As for the experiment at Tokai also, there are more polluting sources in Mito than in Hitachi-Tokai-Katsuta area examined.

Radioactive fallout and natural radioactivity are well known to increase after rainfalls. Therefore, the correlation of mutation data with the rainfall records supplied by the local weather stations was examined for every experiment. However, no evident correlations were found in any experiments between the mutation data and the data of rainfall and of rainy days. Besides this, the greatly period-dependent changes observed in the pink mutation frequency were hardly explained by natural radioactivity, and the geographic differences of the mutation data could not be caused by radioactive fallout, the amount of which must be rather constant in such a limited area as in each experiment. In fact, on the occasion of the largest Chinese nuclear test on September 26, 1976, sudden increases in mutation frequency occurred at all the

points in the Takahama experiment after October 10 (see Table 10), while the detection of ^{131}I at this area was repeatedly reported after October 5 (1.1 and 0.46 pCi/g ^{131}I from leaves of an *Erigeron* species on October 5 and November 4, respectively). Similar but less clear increases were also observed at most of the points in the Hamaoka experiment as seen in Table 4 (the 1976 scoring in Shimane had already been terminated before the effect could be detected).

The mutation data were often found to be inversely related to temperature, as expected from earlier findings (18, 23). By employing the temperature data supplied by local weather stations or by local governmental offices, the correlation of mutation frequency with the average temperature 12 days before the mutation scoring was examined for each experiment [the stamen hairs are most affected genetically 12 days before flowering (4, 5)]. The mutation frequency was generally higher in earlier scoring periods in May to June, decreased gradually with the lowest level in August, and then increased again gradually in later scoring periods in September to October, as typically seen in Maizuru (Tables 10-13). This general pattern was well in accordance with the expectation from the temperature data, namely, temperature being lower in earlier scoring periods, increasing gradually with the highest level in August, and then decreasing gradually in later scoring periods.

However, there were many cases in which mutation frequencies were very much higher than the levels expected from the temperature, as often seen in most of the tables (Tables 2-12, 14-17). Since statistically significant increases in mutation frequency were very often found in the periods of higher temperature (lower mutation frequencies were expected), it is possible to conclude that the temperature variation was not the cause of most of such significant increases of mutations (The high temperature in those periods must have instead suppressed apparent mutation frequencies).

On the other hand, evident relationships were often found between the occurrences of significant mutation increases and the wind data supplied. In Hamaoka, the winds towards the northeast to east (thus southwest to west winds) were conspicuously predominant occupying about 52 to 56% of the wind directed to the land during the scoring periods, and the mutations were significantly increased at the points located to the northeast to east of the nuclear power plant (see Tables 2-5). In Shimane, winds towards the east (east-northeast to east-southeast), southeast (east-southeast to south-southeast) and southwest (south-southwest to west-southwest) constituted about 27-32%, 19-20%, and 32-41%, respec-

tively, of those directed to the land during the scoring seasons but differed greatly in the ratio from period to period, and these wind data seemed to be correlated with the occurrences of significant increases of mutations at various or nonspecific points (see Tables 6-9). In Takahama, the winds towards the south-southwest to south were predominant, constituting around 50% of the winds directed to the land during the scoring seasons. Considering the topography of this area, namely, Mt. Aoba (699 m, often called "Wakasa-Fuji") extended one of its ridges into the site of the nuclear power plant from the southwest, the winds actually directed to the north, south, west, and northwest during scoring periods were calculated to form about 14-19%, 47-50%, 28-32% and 5-6% of the winds directed to the land, respectively. Such distribution of wind directions was also in accordance with the mutation data that significantly higher mutation frequencies were most frequently observed at the points located to the south and west of the nuclear plant (see Tables 10-12).

Correlations of Mutation Data with Nuclear Plants

As described above, only wind records were found to have clear correlations with the mutation increases, out of various environmental factors examined. It suggests strongly that some causative factor(s) of mutation must have been released from the nuclear plants, most probably in gaseous form.

In the Hamaoka experiment in 1974, statistically significant increases in mutation frequency occurred in the period of August 25 to October 5 at the points 1, 4, and 5 located to the northeast to east of the nuclear plant and at the point 6 closest to the reactor (see Fig. 3). The results showed a good coincidence with the test-operation period of the power plant, which was started on August 13 and ceased on October 2 because of a crack found on one of the cooling-system pipes. If we consider the nature of the test employed that the maximum genetic effect of the mutagen treatment appears 12 days later (4), such a coincidence between the mutation increases on the downwind side of the power plant and the test-operation period seems to indicate that the nuclear plant certainly discharged some mutagenic factor(s) during its test operation. In fact, the pooled mutation frequency for the five points at the downwind side for the period from August 25 to October 14 (12 days after starting the test operation to 12 days after stopping it) was found to be significantly increased (see the bottom

line of Table 2). The results obtained from Hamaoka in 1975 were similar but much clearer (see Table 3), probably reflecting the longer test-operation of the reactor in this year, which lasted throughout the scoring period.

Significant increases of mutations that were clearly related to the operation of reaction of reactors were also observed in other experiments. In Takahama, no significant mutation increase was found in 1979 (see Table 13) when both the first and second reactors of the Takahama plant were not in operation during the mutation scoring period, whereas significant increases of mutations were often found in the preceding three years (see Tables 10-12) when at least one of the two reactors was being operated. Also, on comparing mutation frequency between the Takahama and Ohwi experiments in 1979, it is clear that the average frequency was very much higher in Ohwi (compare Tables 13 and 15). In the Ohwi plant, its two huge 1,175-MWe reactors were repeating operations (regular or test) and inspections in this year (being influenced greatly by the Three-Mile Island accident), while no operation at all was done in the Takahama plant during the scoring period.

In Tokai, the average mutation frequency for the whole scoring period at the points near reprocessing facility was highly significantly increased in 1978 (see Table 16) when the reprocessing facility was in operation, while the corresponding frequency was not significantly higher (though it was higher in figure) in 1979 (see Table 17) when the reprocessing was being suspended because of some difficulties.

In some cases, increases in mutation frequency seemed to be correlated with accidental stops of reactors accompanying radioactive releases. Clearly significant increases of mutations were observed at most of the points in Takahama in early to middle July of 1978, following an accidental shut down of the first reactor of the Takahama plant on June 22 (see Table 12). Also, an accidental shut down of the first reactor of the Ohwi plant on July 14 of 1979 was followed by a significant increase of mutations at Oshima in the period of July 23 to August 5 and further followed by mutation increases at both Oshima and Hongo in the next two weeks (see Table 15). Increased mutation frequencies in many of the points in Hamaoka in October of 1976 (see Table 4) may be correlated with the sudden stop of the first reactor of the Hamaoka power plant in later September. Increased mutation frequencies observed in early to middle August of 1979 around the Shimane power plant (see Table 9) could also be correlated to the control-rod accident occurred on July 28.

Most Likely Cause of Increasing Mutations

The above correlations of mutation data with wind direction and with the operation of nuclear plants seem enough to make us suspicious of nuclear plants as the sources of some mutagenic factors.

The factors causative of mutations, which are discharged from nuclear power plants, are of course gaseous radioactive substances. They are predominantly radioactive rare gases such as ^{85}Kr and ^{133}Xe . Since they are exhausted after passing through a charcoal filter, ^{85}Kr possessing a long half-life of 10.3 years becomes the major component. Besides these rare gases, ^{131}I with a half-life of 8.05 days is discharged at rates of 10^{-3} to 10^{-4} of rare gases and is very significant biologically, because it is known that ^{131}I is concentrated from air into plant tissues with an extremely high concentration factor of the order of 10^6 to 10^7 (24, 25). Tritium or ^3H (12.3-year half life), ^{60}Co (5.27-year half life), ^{54}Mn (278-day half life) and many others are also released to the environment together with the above nuclides. All these radioactive nuclides are released also from spent nuclear fuel reprocessing facilities at higher concentrations.

On analyzing the data of environmental radiation level taken by electric power companies and by prefectures concerned, it was found that some increases in the environmental radiation level had indeed occurred around nuclear power plants, especially at Hamaoka. Namely, comparing the data measured with thermoluminescence dosimeters (TLD's) before and after starting the test operation of the Hamaoka plant (the data were available from May of 1972, about two years before the test operation), the environmental radiation level was found to have been increased after starting the test operation as much as 7.5 and 8.7 mR per year in 1974 and 1975, respectively, as the averages for Hamaoka and its two adjacent towns, Omaezaki and Sagara. It is important to note that such increases are the averages for 32 points distributed widely including those more than 7 km apart from the power plant (Comparison of the data before and after starting the test operation for each point is nearly meaningless because the measurements of around 20 mR as accumulated doses for three months were rather close to the limit of accurate measurement with TLD). More important is that the TLD can only measure γ -rays, thus the above data did not include the doses of β -rays. Increases of environmental radiation level around other nuclear power plants were less than 5 millirem per year, according to the published data, satisfying

the publicly guaranteed level of less than 5 mR increase per year. Concerning the environmental radiation levels around the nuclear power complex in Tokai, the data have not been supplied to us, but it was assessed that the maximum increase of the radiation dose due to radioactive gases released around the reprocessing facility would be as high as 32 millirem per year.

Even though the increases of environmental radiation level were small being less than 5, or about 7 to 9 mR, or at most 32 millirem per year, such increases of the dose in the air could be very significant in giving much larger doses to biological tissues. The reasons are as follows. Some of the man-made radioactive nuclides are concentrated greatly in the biological systems (e.g., ^{131}I mentioned above); and the above increases of environmental radiation dose are those of only γ -rays, namely only of external γ -ray dose for living organisms. When radioactive nuclei attach to plants or animals or are incorporated into their tissues, absorbed radiation dose, especially of β -rays, becomes very much larger. Thus the magnitude of increase of the dose absorbed to biological tissues due to concentration of a radioactive nuclide is very much greater than the concentration factor of the nuclide.

Therefore, the increase of environmental radiation level monitored with TLD or other physical instruments represents only a part, probably a minor part, of the actually additionally absorbed dose (external and internal) in biological tissues. Indeed, the increases of pink mutations detected in the stamen hairs of *Tradescantia* were much larger than those expected to occur with the above very small external doses monitored.

Before determining or tracing the most likely cause of increasing mutations, it is necessary to elucidate the responses of *Tradescantia* stamen hairs to chronic exposures to radioactive nuclides with different half lives. When the young inflorescences of KU 9 clone are exposed to a single acute dose of 1 rem, the frequency of pink mutations appearing in the stamen hairs of the mature flowers opening after the exposure changes daily as shown in Figure 7A, with its peak after 12 days [curve drawn based on earlier study (5) with higher doses]. If the 1 rem exposure is repeated daily (or if a 1 rem/day exposure continued) for 5 days, 10 days, or much longer, the resultant mutation frequencies observed will be those obtained by integrating the above curves for the 5, 10, or more days as shown in Figure 7B. Peak values will be observed 14 and 17 (or 18) days after the start of exposure in cases of the 5- and 10-day exposures, respectively, and the frequency will reach a plateau

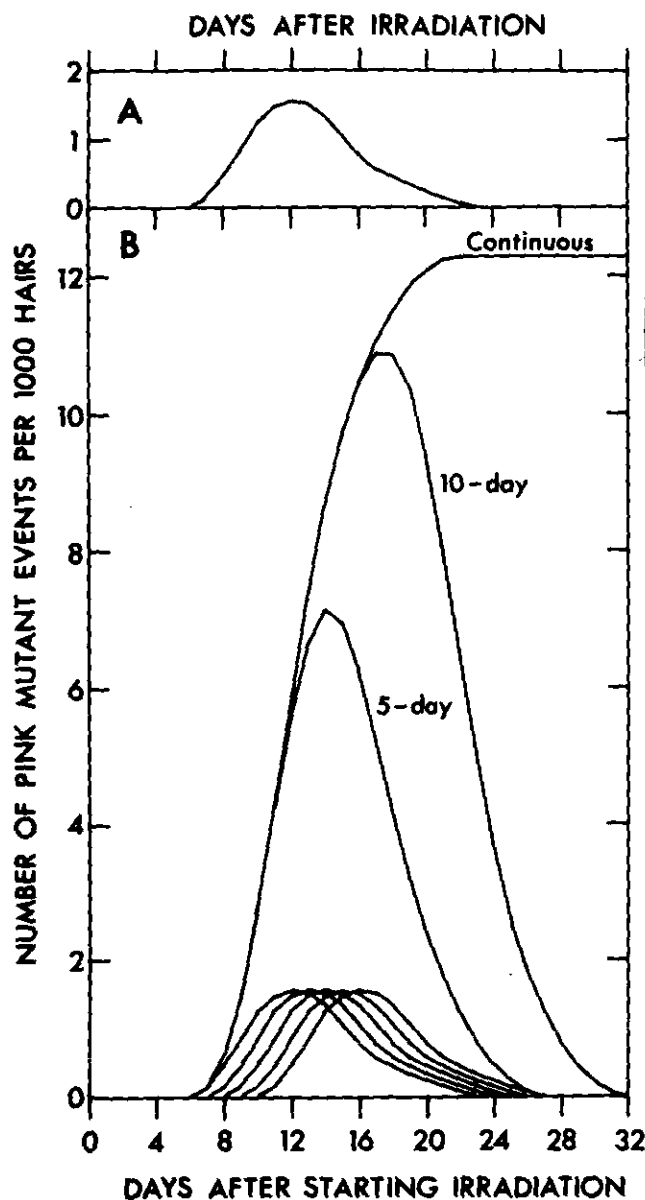


FIGURE 7. Daily changes of pink mutation frequency in the stamen hairs of KU 9 clone expected to occur after (A) a single acute exposure with 1 R and (B) a constant exposure at 1 R/day.

22 days after the start of exposure in case of continuous exposure.

However, when a radioactive nuclide is deposited on young inflorescences, the exposure due to the radioactivity will not be constant because of the decay of the nuclide. For example, if we consider the case of a single ^{131}I deposit which gives 1 rem of exposure on the day of the deposit, the mutation frequency resulted will show a curve as shown in

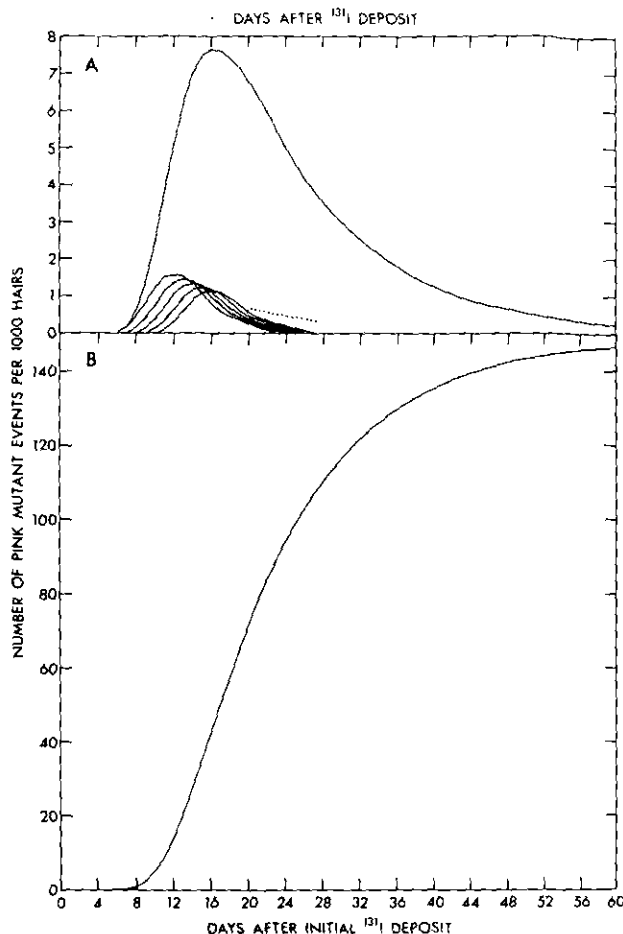


FIGURE 8. Daily changes of pink mutation frequency in the stamen hairs of KU 9 clone expected to occur after (A) a single deposit of ^{131}I giving 1 R on the first day and (B) continuous deposits of ^{131}I of the amount same as in A per day.

Figure 8A, the curve obtainable after integrating the mutation curves induced by the first-day exposure, the second-day exposure, the third-day exposure, and so on (daily exposure decreases to a half every 8.05 days). The peak value appears 16 days after the deposit of ^{131}I in this case (It should be noted that the curve will become closer to that in Figure 7B in cases of other radioactive nuclides with longer half lives). If the above amount of ^{131}I deposit occurs every day for a long period of time, the daily increase in mutation frequency will be observed as shown in Figure 8B. This curve is obtained also by integrating the curves (as shown in Figure 8A) for the daily deposits, and it shows a greatly elevated plateau of mutation frequency to appear after more than 60 days. However, such a constant deposit of ^{131}I does not seem practically occurring, and a combination of a sporadically

intermittent ^{131}I deposits and a constant low-level deposit seems instead more practical. We will observe in that case a repetition of mutation increases as in Figure 8A but with the frequency level gradually rising.

The correlation observed between the occurrence of significantly increased mutation frequency and the operation of nuclear plants indicated that the increases had occurred about 12 days to three weeks after starting operation or about two to four weeks after accidental shut down. Therefore, the most likely major factor causative of mutations seems to be a radioactive nuclide having a relatively short (at least biologically) half life, as that of ^{131}I . The extraordinary high concentration factor of 10^6 to 10^7 of ^{131}I from air into plant tissues (24, 25) seems to give a support to this consideration. The high efficiency of internal exposure from ^{131}I in inducing pink mutations in *Tradescantia* (clone 02) stamen hairs has also been reported, together with the estimated doubling dose of 4 nCi per inflorescence for inducing mutations with ^{131}I (16).

Since a young inflorescence of clone 02 weighs about 1g, the above 4 nCi/inflorescence doubling dose means the ^{131}I concentration of about 4×10^{-3} $\mu\text{Ci/g}$ in the inflorescence. Considering the 10^6 to 10^7 ($\mu\text{Ci}\cdot\text{g}^{-1}$ plant tissue/ $\mu\text{Ci}\cdot\text{cm}^{-3}$ air) concentration factor of ^{131}I mentioned above, it is estimated that around 10^{-9} to 10^{-10} $\mu\text{Ci/cm}^3$ ^{131}I concentration in air is necessary to double the mutation frequency on the assumption that ^{131}I concentrates only directly from air into inflorescence. If we consider the incorporation of ^{131}I from leaves, stems and through roots to be ten times the direct uptake (rather conservative consideration), then 10^{-10} to 10^{-11} $\mu\text{Ci/cm}^3$ concentration of ^{131}I in air would result in the 10^{-3} $\mu\text{Ci/g}$ concentration in inflorescences. However, the ^{131}I concentrations in the air around nuclear power plants are said not to exceed 10^{-13} $\mu\text{Ci/cm}^3$ (the limit of detection). While we can by no means confirm it, since no specific monitoring for ^{131}I has been conducted, the possibility that ^{131}I was the main cause of increasing mutations remains unsolved.

Another possibility concerns with ^3H whose biological significance as internal β -ray source has been also clearly demonstrated in *Tradescantia* stamen hairs (26). Though its half life is much longer than that expected from the above consideration, it seems probable that ^3H is rather quickly washed out after rainfalls. However, ^3H seems less suspected as compared with ^{131}I , since no clear correlation was found between mutation data and rainfall records.

Besides these two nuclides, other radioactive nuclides might have been involved. Our recent study (15) showed that significantly high mutation

frequency in stamen hairs was observed by cultivating *Tradescantia* clone 02 for 76 days in a soil sample taken from the Bikini Island. The soil sample contained 186 ± 9 pCi/g ^{137}Cs , 1.69 ± 0.12 pCi/g ^{60}Co , and some other nuclides. The accumulated external exposures of the inflorescences measured averaged only 62 mR, while the mutation frequency observed corresponded to that induced with about 500 mR. Therefore, it was concluded that internal exposure was more significant than external exposure also for these nuclides. Different radioactive nuclides such as ^{54}Mn , ^{59}Fe , ^{60}Co , ^{90}Sr , ^{95}Zr , ^{95}Nb , ^{137}Cs , and ^{144}Ce have been usually detected in water samples, soils, crops, tea leaves, pine needles, grasses as well as cow's milk around nuclear power plants. For example, ^{144}Ce , ^{137}Cs , ^{95}Nb , ^{95}Zr , ^{90}Sr , and ^{54}Mn were detected in tea leaves collected from Omaezaki (near Hamaoka) in May of 1978 at concentrations of 1.2, 0.15, 0.15, 0.11, 0.071, and 0.0085 pCi/g wet tissue, respectively, together with traces of ^{60}Co and ^{59}Fe . A soil sample from Sagara (also near Hamaoka) collected in October of 1978 was also found to contain 1.2 pCi/g of ^{137}Cs . These various nuclides found in the environment near nuclear power plants might have affected the mutation frequency of *Tradescantia* testers, though the magnitude of the influence might not be large (comparing the figures given above).

Although no clear determination could be made to specify the cause(s) of increasing mutations around nuclear facilities, the correlations of mutation increases with wind direction and with the operation of nuclear plants still cast a strong hint at nuclear plants as the most suspect sources scattering the unspecified mutagen(s).

REFERENCES

1. Nayar, G. G., and Sparrow, A. H. Radiation-induced somatic mutations and the loss of reproductive integrity in *Tradescantia* stamen hairs. *Radiat. Bot.* 7: 257 (1967).
2. Ichikawa, S., and Sparrow, A. H. The use of induced somatic mutations to study cell division rates in irradiated stamen hairs of *Tradescantia virginiana* L. *Japan. J. Genet.* 43: 57 (1968).
3. Ichikawa, S., Sparrow, A. H., and Thompson, K. H. Morphologically abnormal cells, somatic mutations and loss of reproductive integrity in irradiated *Tradescantia* stamen hairs. *Radiat. Bot.* 9: 195 (1969).
4. Ichikawa, S. Relative biological efficiency of 14.1 MeV fast neutrons and ^{137}Cs gamma rays in the stamen hairs of *Tradescantia reflexa* Rafin. *Japan. J. Genet.* 45: 205 (1970).
5. Ichikawa, S. Radiosensitivity of a triploid clone of *Tradescantia* determined in its stamen hairs. *Radiat. Bot.* 12: 179 (1972).
6. Underbrink, A. G., Schairer, L. A., and Sparrow, A. H. *Tradescantia* stamen hairs: a radiobiological test system applicable to chemical mutagenesis. In: *Chemical Mutagens; Principles and Methods for Their Detection*, A. Hollaender Ed., Vol. 3, Plenum Press, New York, 1973, pp. 171-207.

7. Sparrow, A. H., Schairer, L. A. and Villalobos-Pietrini, R. Comparison of somatic mutation rates induced in *Tradescantia* by chemical and physical mutagens. *Mutat. Res.* 26: 265 (1974).
8. Ichikawa, S., and Takahashi, C. S. Somatic mutation frequencies in the stamen hairs of stable and mutable clones of *Tradescantia* after acute gamma-ray treatments with small doses. *Mutat. Res.* 45: 195 (1977).
9. Ichikawa, S., and Takahashi, C. S. Somatic mutations in *Tradescantia* stamen hairs exposed to ethyl methanesulfonate. *Environ. Exptl. Bot.* 18: 19 (1978).
10. Mericle, L. W., and Mericle, R. P. Biological discrimination of differences in natural background radiation level. *Radiat. Bot.* 5: 475 (1965).
11. Nayar, G. G., George, K. P., and Gopal-Ayengar, A. R. On the biological effects of high background radiosensitivity: studies on *Tradescantia* grown in radioactive monazite sand. *Radiat. Bot.* 10: 287 (1970).
12. Ichikawa, S. Somatic mutation rate at low levels of chronic gamma-ray exposures in *Tradescantia* stamen hairs. *Japan. J. Genet.* 46: 371 (1971).
13. Ichikawa, S. Somatic mutation rate in *Tradescantia* stamen hairs at low radiation levels: finding of low doubling doses of mutations. *Japan. J. Genet.* 47: 411 (1972).
14. Sparrow, A. H., Underbrink, A. G. and Rossi, H. H. Mutations induced in *Tradescantia* by small doses of x-rays and neutrons: analysis of dose-response curves. *Science* 176: 916 (1972).
15. Ichikawa, S. and Nagashima, C. Changes in somatic mutation frequency in the stamen hairs of *Tradescantia* grown in the soil samples from Bikini Island, Japan. *J. Genet.* 54: 436 (1979).
16. Tano, S. and Yamaguchi, H. Effects of low-dose irradiation from ^{131}I on the induction of somatic mutations in *Tradescantia*. *Radiat. Res.* 80: 549 (1979).
17. Sparrow, A. H. and Sparrow, R. C. Spontaneous somatic mutation frequencies for flower color in several *Tradescantia* species and hybrids. *Environ. Exptl. Bot.* 16: 23 (1976).
18. Takahashi, C. S. and Ichikawa, S. Variation of spontaneous mutation frequency in *Tradescantia* stamen hairs under natural and controlled environmental conditions. *Environ. Exptl. Bot.* 16: 287 (1976).
19. Ichikawa, S., Nauman, C. H., Sparrow, A. H., and Takahashi, C. S. Influence of radiation exposure rate on somatic mutation frequency and loss of reproductive integrity in *Tradescantia* stamen hairs. *Mutat. Res.* 52: 171 (1978).
20. Ichikawa, S. and Nagata, M. Increase of mutation frequency in *Tradescantia* at the surrounding area of the Hamoka nuclear power plant. *Kagaku* 45: 417 (1975).
21. Ichikawa, S. Effects of low-level radiations on living organisms. *Chem. Today* 59: 22 (1976).
22. Grossman, K. The spiderwort: radiation monitor. *Garden* 3 (3): 22 (1979).
23. Yamashita, A. *Tradescantia* in the studies of genetic effects of low level radiation. *Hoken-Butsuri* 11: 263 (1976).
24. Marter, W. L. Radioiodine release incident at the Savannah River plant. *Health Phys.* 9: 1105 (1963).
25. Soldat, J. K. The relationship between ^{131}I concentrations in various environmental samples. *Health Phys.* 9: 1167 (1963).
26. Nauman, C. H., Klotz, P. J., and Schairer, L. A. Uptake of tritiated 1,2-dibromoethane by *Tradescantia* floral tissues: relation to induced mutation frequency in stamen hair cells. *Environ. Exptl. Bot.* 19: 201 (1979).